

Exhibit 1

# nature

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Roaring by red deer stags may attract females, but it also advances the date of oestrus in hinds. Playback experiments (page 648) provide the first evidence that male vocalizations can affect the timing of ovulation in female mammals. (Photograph: Steve Albon.)

## THIS WEEK

### Superconductivity

A specific heat anomaly may provide important clues to the nature of high-temperature superconductivity, see pages 607 and 601. For an update on both the theoretical and experimental results in superconductivity see page 611.

### Flame convection

Flames, normally wayward under zero gravity conditions,



can be controlled by the application of electronic fields, page 635.

### New messengers

The role of inositol tetrakisphosphate (Ins P<sub>4</sub>) in intracellular calcium regulation, page 653, and a possible extracellular action of Ins P<sub>4</sub> and Ins P<sub>3</sub>, page 656.

### Retinoic-acid receptor

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### Septic shock

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### Flower colour

By a combination of classical and molecular genetics the metabolic pathway for the



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in brackets was unity. To conform with the generalized second law, as above,  $(N/2)^{1/4} C \leq 8/3$ ; the factor of two arises because the value of  $a$  already takes into account the two photon polarization states. The number  $N$  also enters into the evaporation time  $t_e = (640/27\pi)\kappa^2 M_{\text{th}}^3$ , so that the exponent of the RW function at the matching time is

$$\sqrt{\mu} t_e = C^2 (2N)^{-1/2} \left(\frac{a}{12}\right)^{1/2} \frac{640}{27\pi} (\kappa M_{\text{th}}^2)^{1/2} \quad (24)$$

This expression clearly depends on the supplementary relation between  $C$  and  $N$  in ways which are now being investigated.

Another refinement to the model would include the fact that the constants  $\alpha$  and  $\beta$  are time-dependent because the cosmological model will go through a series of temperature thresholds associated with the masses of the various constituents. Above each threshold, the appropriate particle is effectively massless, and contributes to the value of  $\beta$ , but below the threshold they become massive and contribute instead to  $\alpha$ .

Our oversimplified model omits many potential complications, such as CP-violation and the annihilation of matter and antimatter. But we are reluctant to introduce extra mechanisms as they would require new parameters destroying the predictive power of the model. Of course, it may be that the numerical agreements we find are coincidental, but there is also the challenging possibility that our model can be used to relate typical

constants, such as those appearing in grand unified theories, to the fundamental constants  $h$ ,  $c$  and  $\kappa$ . This is being explored at present.

It should be noted finally that because non-conformal fluctuations in the Minkowski vacuum contribute positively to the energy-momentum balance, as shown above, they stabilize the vacuum. The more homogeneous the initial spacetime, the more probable is the occurrence of an instability and subsequent transition to a self-consistent cosmology. The cosmological principle, that the Universe is homogeneous and isotropic, thus seems to be closely related to the mechanism we have described here. There is another intriguing problem. As RW expansion proceeds, the universe becomes more dilute, and tends to a vacuum-like configuration. The conditions for instability would therefore seem to reappear beyond some definite threshold of energy density. This would lead to a resurgence of the vacuum to inflationary universe transition. Strangely enough, in the case of a spatially open universe, we would encounter a type of repetition, reminiscent of the spatially closed case. The steady-state cosmology unexpectedly reappears, but on a new, huge scale. The main problem to be addressed here is the instability condition for a sufficiently dilute universe.

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## Identification of a receptor for the morphogen retinoic acid

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*Analysis of complementary DNA encoding a novel gene product reveals striking similarity to the steroid and thyroid hormone receptors. Binding and transcription activation studies show it to be a receptor for the vitamin A-related morphogen retinoic acid.*

A CENTRAL problem in eukaryotic molecular biology is the elucidation of molecules and mechanisms that mediate specific gene regulation in response to exogenous inducers such as hormones or growth factors. Steroid receptors are intracellular proteins that mediate complex effects on development, growth and physiological homeostasis by selective modulation of gene transcription. The recent cloning of the genes for the human glucocorticoid<sup>1</sup> and oestrogen<sup>2</sup> receptors has allowed a detailed biochemical characterization and revealed that these molecules contain discrete DNA-binding and ligand-binding domains<sup>3-7</sup>.

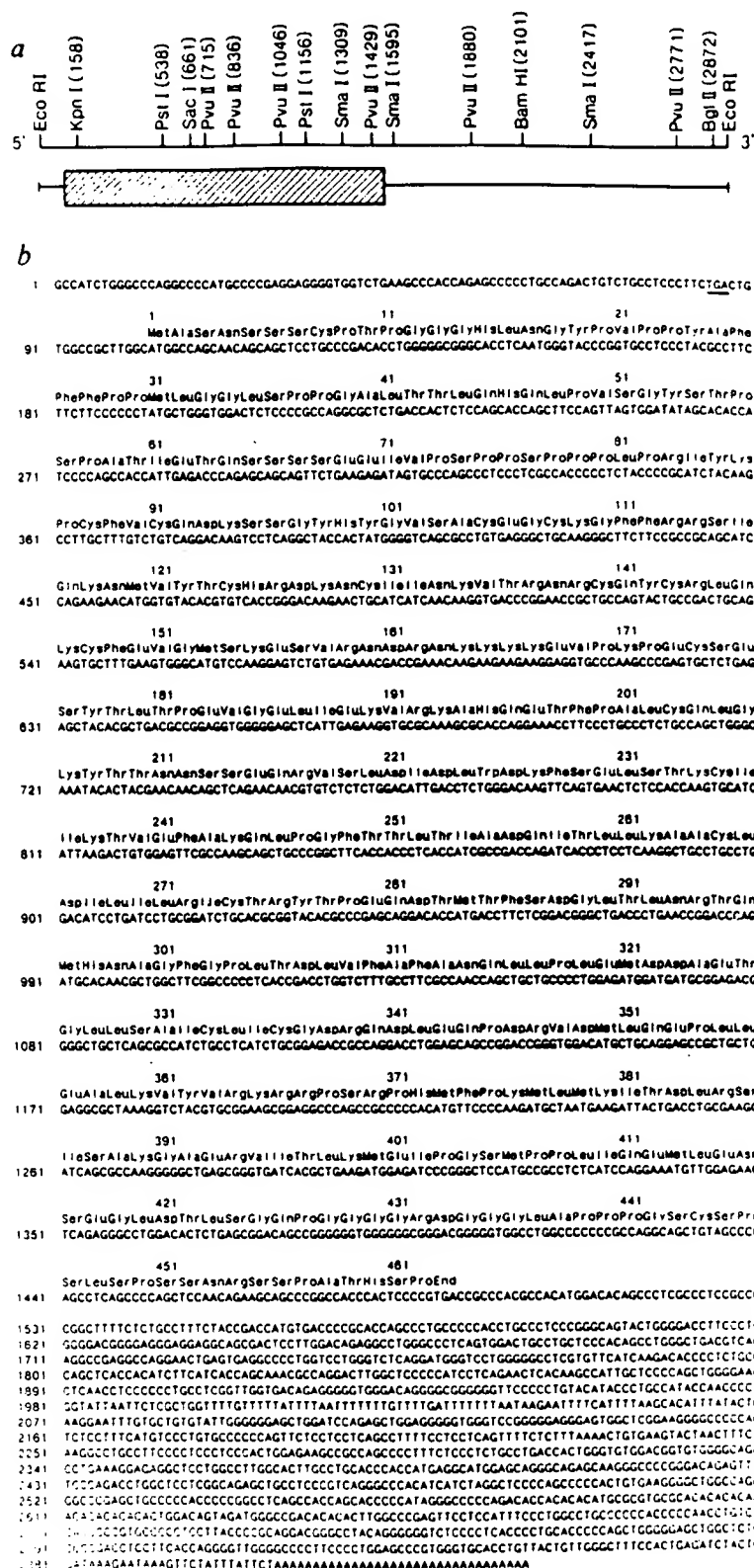
Sequence analysis of the human glucocorticoid receptor gene revealed similarity with the product of the *v-erbA* oncogene of avian erythroblastosis virus (AEV)<sup>8</sup>. We and others subsequently demonstrated the cellular homologue of *v-erbA* to be the thyroid hormone receptor<sup>9,10</sup>. Like the steroids, thyroid hormones mediate important developmental events and control metabolism in the adult. Apparently, the common structure of their receptors is reflected in the analogous action of the hormones.

The discovery that the DNA-binding domain of the steroid and thyroid hormone receptors is highly conserved led to the proposal that this segment might be diagnostic for related ligand-inducible transcription factors. It might also be possible to use the DNA sequences encoding these domains as hybridization probes to scan the genome for related, but novel, ligand receptors. Using this approach, we have identified several new gene products. One has recently been shown to be the human aldosterone receptor<sup>11</sup>; a second is a novel thyroid hormone receptor expressed at high levels in the central nervous system<sup>12</sup>. In this report we describe the isolation and characterization of a cloned full-length cDNA encoding a 462 amino-acid polypeptide with similarity to the DNA-binding and ligand-binding domains of the steroid and thyroid hormone receptors.

We have devised a new and potentially general strategy to determine the functional ligand for this receptor. This strategy takes advantage of the modular structure of the steroid receptor and the proposal that functional domains may be interchangeable.

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**Fig. 1** DNA and primary amino-acid sequence of  $\lambda$ hKIR. *a*, Schematic representation and restriction enzyme map of the  $\lambda$ hKIR clone. The stippled box represents the predicted open reading frame. *b*, The complete nucleotide sequence of  $\lambda$ hKIR is shown with the predicted amino-acid sequence given above the long open reading frame. An upstream in-frame stop codon at nucleotides 35-37 and the putative polyadenylation signal are underlined.

**Methods.** A 63-mer oligonucleotide corresponding to nucleotides 408-477 of the genomic sequence published by Dejean *et al.*<sup>13</sup> was used as a hybridization probe to screen a human testis  $\lambda$ gt10 library. The hybridization mixture contained 35% formamide, 1 $\times$  Denhardt's 5 $\times$  SSPE (0.15 M NaCl, 0.01 M Na<sub>2</sub>HPO<sub>4</sub>, 0.001 M EDTA), 0.1% sodium dodecyl sulphate (SDS), 100  $\mu$ g ml<sup>-1</sup> denatured salmon sperm DNA and 10<sup>6</sup> c.p.m. ml<sup>-1</sup> of <sup>32</sup>P-labelled oligonucleotide. Duplicate nitrocellulose filters were hybridized at 42°C for 16 h, washed three times for 20 min each in 2 $\times$  SSC, 0.1% SDS (1 $\times$  SSC is 150 mM NaCl, 15 mM sodium citrate) at 55°C and autoradiographed at -70°C with an intensifying screen. Clone  $\lambda$ HT1R obtained from this screening was partially characterized and then used as a hybridizing probe to screen a human kidney  $\lambda$ gt10 cDNA library<sup>37</sup>. For this screening, the washing conditions were modified to 1 $\times$  SSC with 0.1% SDS at 68°C. Several cDNA clones were isolated and the longest clone,  $\lambda$ hK1R, was digested with a number of restriction enzymes and the resulting fragments were subcloned in both orientations into the M13 sequencing vectors mp18 and mp19 and sequenced by the dideoxy procedure<sup>38</sup>. DNA sequences were compiled and analysed by the programs of Devereux *et al.*<sup>39</sup> and Staden.<sup>40</sup>

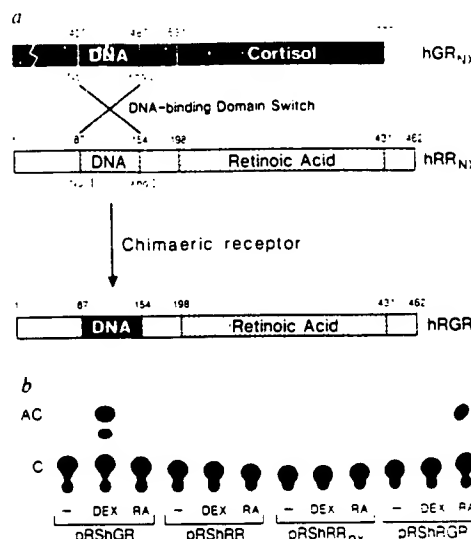
These data identify a presumptive specific high-affinity retinoic-acid receptor. The homology of this receptor to those of the steroid and thyroid hormones suggests a unifying hypothesis for both receptor structure and hormone action.

### Candidate receptor

Analysis of the integration site of a hepatitis B virus from a human hepatocellular carcinoma led to the fortuitous iden-

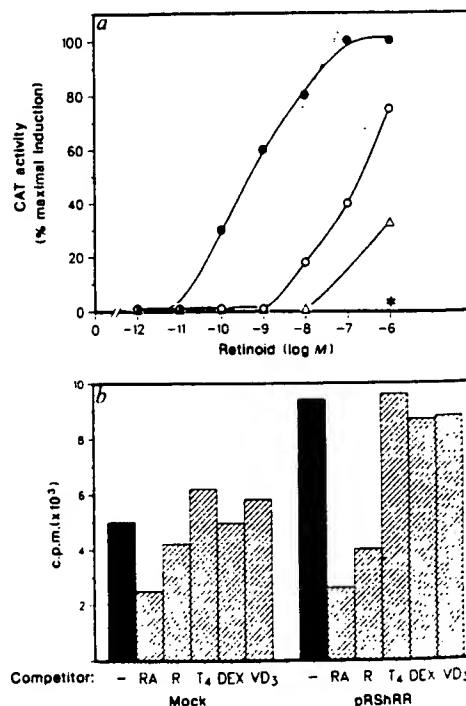
**Fig. 2 a.** Construction of the chimaeric receptor hRGR. The domain-structure of the various constructions are shown schematically, the numbers correspond to the amino-acid positions of each domain. The DNA-binding domains are represented by 'DNA' and the ligand-binding domains by their respective inducers. The *NotI* and *XhoI* sites created by site-directed mutagenesis to permit the exchange of the DNA-binding domains between receptors are indicated. **b.** Induction of CAT activity by retinoic acid. The expression vectors were cotransfected into CV-1 cells with the reporter plasmid MTVCAT and cultured for 2 days in absence or presence of 100 nM dexamethasone (DEX) or retinoic acid (RA). The receptors inserted into the expression vectors are: pRShGR, human glucocorticoid receptor; pRShRR, human retinoic-acid receptor; pRShRR<sub>NA</sub>, mutated human retinoic-acid receptor with *NotI* and *XhoI* sites; pRShRGR, chimaeric receptor composed of the human retinoic-acid receptor which DNA-binding domain has been replaced by the human glucocorticoid receptor DNA-binding domain.

**Methods.** **a.** Restriction enzyme fragments of the cDNA inserts of  $\lambda$ hK1R and hGR<sup>1</sup> were subcloned into the *KpnI* and *BamHI* sites of the mp19 vector and mutagenized according to the method of Kunkel<sup>41</sup>. The oligonucleotides used for the creation of the *NotI* site within hGR and hRR were 28 and 31 nucleotides respectively, whereas the oligonucleotides used for the creation of the *XhoI* site within hGR and hRR were 24 and 23 nucleotides. The creation of the *NotI* site resulted in the mutation of Pro 416 to an Arg residue in hGR<sub>NA</sub>, and in the mutation of Ile 84 and Tyr 85 to Pro residues in hRR<sub>NA</sub>. The introduction of the *XhoI* site did not alter the hGR<sub>NA</sub> amino-acid sequence but resulted in the mutation of Lys 155 to a Leu residue in hRR<sub>NA</sub>. The mutant receptors were then transferred to the expression vector pRS<sup>1</sup>, and the *NotI*-*XhoI* restriction fragment of pRShGR<sub>NA</sub> containing the hGR DNA-binding domain was introduced into pRShRR<sub>NA</sub> between the *NotI* and *XhoI* sites to create pRShRGR. **b.** Cell transfection and CAT assay. The recombinant DNA constructs (5  $\mu$ g each) were introduced into CV-1 cells by calcium phosphate coprecipitation<sup>42</sup>. The cells were cultured for two days in serum-free media supplemented with Nutridoma (Boehringer Mannheim) in presence or absence of inducers. CV-1 cells were then prepared for CAT assays as described<sup>43</sup> and the assays performed for 3 h using 25  $\mu$ g of protein extract. Experiments with retinol were conducted in subdued light.



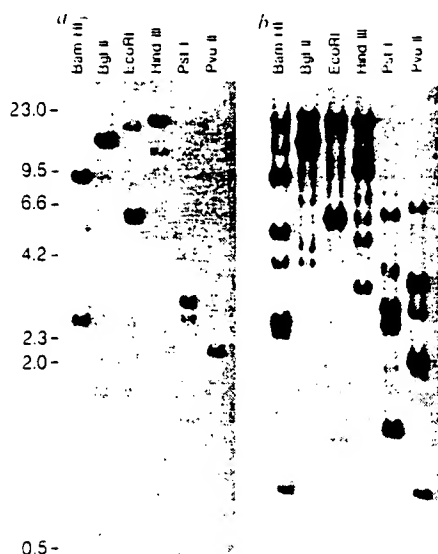
**Fig. 3 a.** Dose-response to retinoids. CV-1 cells cotransfected with pRShRGR and pMTVCAT were treated with increasing concentrations of retinoids or a single 1  $\mu$ M dose (\*) of testosterone, dihydrotestosterone, oestrogen, cortisol, aldosterone, progesterone, triiodo-thyronine (T<sub>3</sub>), thyroxine (T<sub>4</sub>), dihydroxy-vitamin D<sub>3</sub> (VD<sub>3</sub>) and 25-OH-cholesterol. The levels of CAT activity were plotted as percentages of the maximal response observed in this experiment. ●, Retinoic acid; ○, retinol; △, retinyl acetate or retinyl palmitate. **b.** Retinoic acid binding to cytosol extracts of transfected COS-1 cells. Bars represent bound <sup>3</sup>H-retinoic acid determined in absence (black bars) or presence (stippled bars) of a 1,000-fold excess of various competitors. The values represent the mean of quadruplicate determinations. Competitors are retinoic acid (RA), retinol (R), T<sub>4</sub>, dexamethasone (DEX) and VD<sub>3</sub>.

**Methods.** **a.** CV-1 cell cotransfections and CAT assays were performed as described in Fig. 2. Retinoic acid was dissolved in a minimum volume of dimethyl sulphoxide and diluted in ethanol. All other products were diluted in ethanol and control cultures received 0.1% solvent (v/v) in media. Dose-response curves were performed in triplicate. **b.** Subconfluent COS-1 cells were transfected with 10  $\mu$ g per dish of a control plasmid (pRS) or pRShRR by the DEAE-Dextran method<sup>44</sup>. Cells were maintained for 2 days in Dulbecco's minimal Eagle's medium (DMEM) with 5% charcoal-treated fetal calf serum, then harvested in TNE (40 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA) and lysed by Dounce homogenization in hypotonic buffer (50 mM Tris-HCl pH 7.4, 0.1 mM EDTA, 5 mM dithiothreitol, 10 mM NaMoO<sub>4</sub>, 10% glycerol, 0.5 mM phenylmethylsulphonyl fluoride) and centrifuged at 100,000g for 30 min to yield the cytosol fraction. Incubations were performed in hypotonic buffer with 150  $\mu$ g of protein from the cytosolic fraction and  $2 \times 10^{-8}$  M <sup>3</sup>H-retinoic acid (NEN, 52.5 Ci per mmole) in a total volume of 200  $\mu$ l. Specific binding was measured by the addition of  $2 \times 10^{-5}$  M of competitors. Reactions were carried out at 4 °C for 16 h. Bound <sup>3</sup>H-retinoic acid was measured using DE-81 filters. Reactions were placed on filters for 1 min, rinsed with 5 ml of washing buffer (50 mM Tris-HCl pH 7.4, 0.1 mM EDTA, 0.1% Triton X-100), dried and counted by liquid scintillation spectrophotometry.



**Fig. 4 S** placenta. After separation per lane hybridization encompassing 0.1% SD in 0.1% (size in of hum. non-stru sample) used.

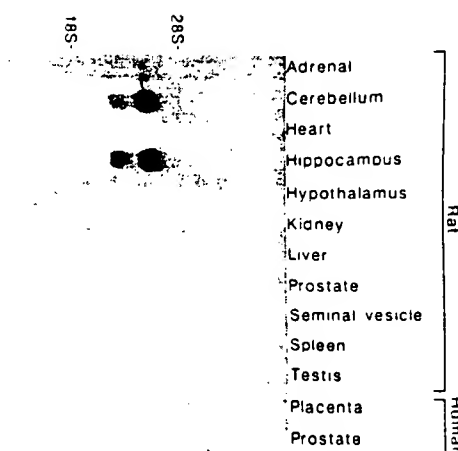
tification to the DN. To exami unknown sequence cDNA lib a testis (AhtIR). Agt10 kil clone (AhtIR) reveals a with a pr to nucleo to ATG ag: translati terminate Another conform terminate 3'-untran (AATAA tract<sup>15</sup>. A poly is encode of the pr in vitro tr to corres Amino-a glucocor degree o amino ac that this



**Fig. 4** Southern blot analysis of human genomic DNA. *a*, Human placenta DNA was digested with the indicated restriction enzymes. After separation of the digested DNA in a 0.8% agarose gel (10 µg per lane) and transfer to nitrocellulose filters<sup>45</sup>, the blots were hybridized with an *EcoRI*-*PvuII* fragment from  $\lambda$ hTIR (~600 bp) encompassing the DNA-binding domain of the hRR under high stringency conditions (50% formamide, 5× SSPE, 1× Denhardt's, 0.1% SDS, 100 µg ml<sup>-1</sup> salmon sperm DNA). The filter was washed in 0.1× SSC, 0.1% SDS at 65°C. The  $\lambda$  *HindIII* DNA markers (size in kb) are aligned to left of the autoradiograph. *b*, Analysis of human placenta DNA using the same probe as in *a* under non-stringent conditions. A parallel blot containing identical samples was hybridized as in *a*, except that 35% formamide was used. The filter was washed in 2× SSC, 0.1% SDS at 55°C.

ification of a novel genomic sequence with striking similarity to the DNA-binding domain of the steroid hormone receptors<sup>13</sup>. To examine the possibility that this gene encodes a previously unknown receptor, an oligonucleotide derived from this sequence was labelled and used to probe a number of human cDNA libraries. Five positive clones were initially isolated from a testis cDNA library. The insert from one of these clones ( $\lambda$ hTIR) was used to isolate additional cDNA clones from a  $\lambda$ gt10 kidney cDNA library. A restriction map of the largest clone ( $\lambda$ hKIR) is shown in Fig. 1*a*. Nucleotide sequence analysis reveals a long open reading frame of 462 amino acids beginning with a presumptive initiator methionine codon corresponding to nucleotides 103–105 (Fig. 1*b*). The sequence surrounding this ATG agrees with the consensus described by Kozak<sup>14</sup> for a translation initiation site. Upstream of the ATG is an in-frame terminator providing support for the initiator methionine. Another methionine found 30 codons downstream does not conform to the consensus and is an unlikely initiator. After the terminator codon at position 1,489–1,491 is a 1,419 nucleotide 3'-untranslated region with a consensus polyadenylation signal (AATAAA) found 20 nucleotides upstream of a polyadenylated tract<sup>15</sup>.

A polypeptide of relative molecular mass 50,772 (*M*<sub>r</sub> 51K), encoded within the translational open reading frame. The size of the protein encoded by the insert of  $\lambda$ hKIR was verified by *in vitro* translation of RNA<sup>16</sup> derived from this insert and found to correspond to the predicted size of 54K (data not shown). Amino-acid sequence of this protein has been compared to the glucocorticoid and thyroid hormone receptors. The highest degree of similarity is found in a cysteine-rich sequence of 66 amino acids beginning at residue 88. We have previously shown that this region of the hGR is the DNA-binding domain<sup>3,4</sup>. In



**Fig. 5** Northern blot analysis of retinoic acid receptor mRNA in rat and human tissues.

**Methods.** Total RNA was isolated from various tissues using guanidine thiocyanate<sup>46</sup>, 20 µg were separated on a 1% agarose-formaldehyde gel, transferred to a nitrocellulose filter, and hybridized under stringent conditions using the probe described in Fig. 4. Ribosomal RNAs (28S and 18S) are indicated for size markers. The nitrocellulose filter was autoradiographed at -70°C with an intensifying screen for 1 week.

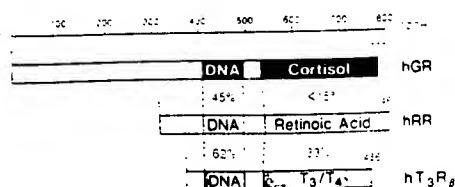
addition, mutagenesis and expression studies have provided direct evidence for its role in transcriptional activation of genes harbouring glucocorticoid response elements (GREs)<sup>3,4</sup>.

### Ligand identity

As the ligand for the gene product of  $\lambda$ hKIR was unknown, we wished to develop a quick and sensitive assay to reveal its identity. The DNA-binding domains of the human glucocorticoid and oestrogen receptors can be interchanged, yielding functional hybrid receptors. One such chimera recognizes the glucocorticoid-responsive element of the MMTV-LTR but stimulates transcription in an oestrogen-dependent fashion<sup>5</sup>. This suggests a general strategy that can be exploited to identify the ligand-binding properties of a novel hormone receptor. To test this approach we have substituted the DNA-binding domain of the  $\lambda$ hKIR gene product with the DNA-binding domain from the hGR (Fig. 2*a*). The assay system is established by transfecting CV-1 cells with the hybrid receptor gene and a MMTV-CAT reporter gene. Transfected cells are then systematically challenged with a battery of candidate ligands and induction monitored by changes in chloramphenicol acetyltransferase (CAT) activity. Because of their hormone-like activities, the retinoids, including retinol (vitamin A) and retinoic acid, were evaluated as potential inducers. Remarkably, retinoic acid elicited a dramatic increase in CAT activity of the hybrid receptor (Fig. 2*b*). No effect upon CAT activity was observed using the parent vector, pRShRR<sub>nx</sub>, or the wild-type gene product from  $\lambda$ hKIR, here referred to as the human retinoic acid receptor (hRR). As expected, the hybrid receptor is not induced by glucocorticoids, and the hGR is not induced by retinoic acid.

As shown in Fig. 3*a*, retinoic acid gives an ED<sub>50</sub> value of  $6 \times 10^{-10}$  M on CAT activity induced by the hybrid receptor, which is consistent with ED<sub>50</sub> values observed for retinoic acid in a variety of biological assays<sup>17</sup>. Retinol functions as a weak agonist with an ED<sub>50</sub> value greater than 100 nM. Retinyl acetate and retinyl palmitate are even weaker inducers. A number of natural and synthetic ligands including testosterone, dihydrotestosterone, oestrogen, dexamethasone, cortisol, aldosterone, progesterone, T<sub>3</sub>, T<sub>4</sub>, Vitamin D<sub>3</sub> and 25-OH-cholesterol did not induce CAT activity.

Our hybrid receptor activation assay allows us to screen a large number of potential activators, but does involve the use



**Fig. 6** Schematic amino-acid comparisons of the hGR, hRR and hT<sub>1</sub>R<sub>3</sub> structures. Amino-acid sequences have been aligned schematically with the percentage amino-acid identity for each region of similarity in the intervals between dotted lines.

of a hybrid protein. To corroborate the identity of the  $\lambda$ hK1R gene product as the retinoic acid receptor, the binding properties of the expressed protein were evaluated following transfection of COS-1 cells. As shown in Fig. 3b, transfected cells reveal increased capacity to bind [ $^3$ H]retinoic acid. This increase occurs over an endogenous background that is probably a consequence of the presence of cellular retinoid-binding proteins (see discussion) as well as nonspecific binding. Consistent with the activation studies, the binding can be completely inhibited by competition with unlabelled retinoic acid but only partially by retinol. Thyroid hormones, dexamethasone and vitamin D<sub>3</sub> did not compete with the binding of retinoic acid.

## A gene family

To determine whether this gene is unique and to identify potentially related genes, human DNA was examined by Southern blot analysis. Hybridization of restriction endonuclease-digested human DNA with a labelled DNA fragment derived from the coding region of the hRR polypeptide produced three bands in every digestion consistent with a single hybridizing genetic locus (Fig. 4a). This hybridization pattern is unrelated to the restriction endonuclease map described by Dejean *et al.* for the hepatitis B virus pre-integration site<sup>13</sup>. When the hybridization conditions were relaxed, however, additional bands were observed in the products of each enzyme digestion (Fig. 4b). These observations indicate that there is another locus, and possibly more, in the human genome related to the retinoic-acid receptor.

### Expression of the hRR gene

Because retinoic acid is known to exert effects on a large number of different cell types, we examined the expression of the hRR gene. Total cytoplasmic RNAs isolated from a variety of rat and human tissues were size-fractionated and transferred to a nitrocellulose filter. Hybridization with a 600-base pair (bp) restriction fragment from *hTIR* reveals a major RNA species of 3,200 nucleotides with highest levels in the hippocampus, adrenals, cerebellum, hypothalamus and testis (Fig. 5). Longer exposure shows that most tissues contain a small amount of the 3.2-kilobase (kb) transcript, although it is undetectable in some tissues such as liver. The size of the messenger RNA indicates that we have isolated a nearly full-length hRR cDNA.

## Conclusions

The data in this report identify the gene product of  $\lambda$ hK1R as the human retinoic-acid receptor based on three criteria. First, the overall structural similarity of the hRR to steroid and thyroid hormone receptors (Fig. 6) suggests that it is likely to be a ligand-responsive regulatory protein. Second, an expressed chimaeric receptor, consisting of the hGR DNA-binding domain and the presumptive ligand-binding domain of the hRR, can act as a transcriptional regulator of a glucocorticoid-inducible reporter gene only in response to retinoic acid. This induction occurs at physiological levels. Third, expression of the candidate hRR in transfected cells selectively increases the capacity of those cells to bind retinoic acid.

**Development and oncogenesis.** The retinoids comprise a group of compounds including retinoic acid, retinol (vitamin A) and a series of natural and synthetic derivatives that together exert profound effects on development and differentiation in a wide variety of systems<sup>18-22</sup>. Although early studies focused on the effects of retinoids on epithelial growth and differentiation, the actions have been shown to be more widespread than previously suspected. Many recent studies have examined the effects of these molecules on a variety of cultured cell lines including neuroblastomas<sup>23</sup>, melanomas<sup>24</sup> and fibroblasts<sup>25</sup>. In the human promyelocytic leukaemia cell line, HL-60, retinoic acid is a potent inducer of granulocyte differentiation<sup>26</sup>. In F9 teratocarcinoma stem cells, retinoic acid will induce the differentiation of parietal endoderm, characteristic of a late mouse blastocyst<sup>27-29</sup>. Retinoic acid has been shown to exert equally potent effects in development. For example, in the developing chick limb bud, retinoic acid substitutes for the action of the polarizing region in establishing the anterior-posterior axis<sup>30</sup>. By controlling exposure to retinoic acid, it is possible to generate novel patterns of limb structure. Although retinoic acid is primarily considered a morphogen, Northern blot analysis suggests a re-evaluation of its function in the adult. In humans, retinoid deficiency has been linked to an alarming increase in a variety of cancers<sup>31</sup>. Retinoids have also been shown to inhibit tumour progression in animals and block the action of tumour promoters *in vitro*. In this context, the hRR may be considered as a negative regulator of oncogenesis.

The identification of a cellular retinol-binding protein (CRBP) and a distinct cellular retinoic-acid binding protein (CRABP) in the mid-1970s led to a proposal that they might represent specific intracellular receptor systems (see ref. 32 for review). These molecules are relatively small (134 amino acids for CRBP) and bear no obvious structural homology to the steroid receptors. More importantly, they show no similarity to the retinoic-acid receptor identified in this manuscript. Despite a detailed biochemical characterization and their recent cloning<sup>32-36</sup>, there is no direct evidence that establishes a decisive role for CRBP and CRABP as mediators of retinoid action.

**A superfamily of regulatory genes.** Two surprising results have emerged from the studies presented here. The first is the discovery of a family of retinoic-acid receptor-related genes, implying the existence of one or more other proteins with closely related properties. Physiological studies demonstrate that both retinoic acid and retinol (vitamin A) can exert potent effects on cellular differentiation and that these effects are often not linked. It thus seems likely that at least one related gene product might be a specific retinol receptor or a receptor for another member of the retinoid family. The second surprising observation from these results is the close kinship of the retinoid receptor with the thyroid hormone receptor. This relationship is surprising in part because of the structural dissimilarity of the thyroid hormones and the retinoids. Thyroid hormones are derived from the condensation of two tyrosine residues whereas the retinoids are derived from mevalonic acid. The observation that chemically distinct molecules interact with receptors sharing common structures probably reflects a common mode of action with which they elicit their particular regulatory effects. Based on this analogy, we can now propose that the interaction of retinoids with their intracellular receptors induces a cascade of regulatory events that results from the activation of specific sets of genes by the hormone/receptor complex. Although animals employ diverse means to control their development and physiology, the demonstration that the retinoic-acid receptor is part of the steroid receptor superfamily suggests mechanisms controlling morphogenesis and homeostasis may be more universal than previously suspected.

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## LETTERS TO NATURE

### How accurate were seventeenth-century measurements of solar diameter?

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The assumption that the solar diameter is constant over periods that are short compared to the nuclear process has recently been questioned. In 1979 Eddy and Boornazian claimed that measurements of the solar diameter made at the Greenwich and US Naval observatories show a decrease of  $\sim 2$  arcs, or 0.1%, per century over the period 1836-1953 (refs 1, 2). Others have since extended this baseline by studying the evidence from Mercury transits<sup>3</sup> and assessing the entire range of evidence from the beginning of the eighteenth century to the present<sup>4</sup>, and have found little or no evidence for a secular change in the solar diameter. Most recently, Ribes *et al.*<sup>5</sup> have pushed the time baseline back to 1666, by considering the micrometer and transit measurements of the solar diameter made in Paris by Jean Picard (1666-82) and Philippe and Gabriel-Philippe de La Hire (1683-1719). Not only are these observations of importance for the investigation of solar changes at the end of the Maunder minimum, they can also be used for comparison with modern values. We discuss here the accuracy of Jean Picard's micrometer measurements of the solar diameter and his successors' transit measurements in light of the claim by Ribes *et al.*<sup>5</sup> that these data provide evidence for a change in the apparent size of the Sun at the end of the Maunder minimum and a difference from the current size. Our evidence suggests that the necessary corrections to the measurement of sizes with early telescopes are larger than Ribes *et al.* assume. Therefore we call into question their conclusion that the Sun rapidly changed in size during this period.

Ribes *et al.*<sup>5</sup> argue that their analysis of the measurements of Picard and Philippe and Gabriel-Philippe de la Hire shows that

the observed solar diameter, uncorrected for systematic errors, was  $32' 9''$  for the period 1666-82, and then decreased smoothly to  $32' 6''$  over the period 1683-1718. The present-day value is  $31' 59.3''$  (ref. 6). The 1666-82 period corresponds to the deep Maunder minimum and the 1683-1718 period to the end of the Maunder minimum; micrometer measurements make up the bulk of the data from the former, while transit measurements constitute the data from the latter period. While the accuracy of recent observations can be assessed with some precision, the accuracy of older observations presents severe problems.

Jean Picard (1620-82) was a pioneer in the precision astronomy made possible by the micrometer and the application of telescopic sights to measuring arcs. Picard's measurements of the diameters of the Sun, Moon and planets, begun in 1666, were the first sustained series of micrometer measurements made. They survive in manuscript form in the Paris Observatory and were published in 1741 in P-C Le Monnier's *Histoire Céleste*<sup>7</sup>. In assessing the accuracy of these measurements, two aspects must be considered, the quality of the image and the accuracy of the micrometer.

The quality of the image will significantly affect the measurement of solar diameter, regardless of whether the image was projected or viewed directly through dark glass. The image formed is a product of the observing environment and the telescope itself. Each component will produce an essentially circular disk (ignoring lens astigmatism) with a peak surface brightness at the middle and a monotonically decreasing surface brightness with an approximately gaussian distribution. There are several ways of characterizing such an image, the most convenient being the full width at half the maximum intensity (FWHM). If the image is made up of more than one component, each component will add quadratically to the total; for example, two equal components would increase the resultant image size by a factor of 1.414. The observing environment produces the phenomenon of astronomical seeing, occasioned by inhomogeneities in the refractive index of the air along the line of sight, caused by small variations in temperature. Very small



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